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# Effectiveness of Thirteen Vertebrate Repellents as Rodent Trigeminal Stimulants

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MASON, J. R., D. L. NOLTE AND B. P. BRYANT. *Effectiveness of thirteen vertebrate repellents as rodent trigeminal stimulants.* PHYSIOL BEHAV **60**(6) 1449–1452, 1996.—Repellent chemicals are presumed to activate trigeminal neurons, including polymodal nociceptors, but few data are available that bear on this notion. In the present experiment, we assessed multi-unit and single-unit responses of neurons in the rat lingual trigeminal nerve to 13 candidate repellents and a thermal stimulus. All of the chemicals evoked trigeminal responses, and neural activity was predictable from available behavioral data. These results are consistent with the view that repellents are irritants. The results also suggest that electrophysiological methods may represent a useful method for screening candidate repellent compounds. *Copyright © 1996 Elsevier Science Inc.*

Chemosensory Electrophysiology Irritation Rat Repellent Trigeminal

AVOIDANCE of repellent chemicals is frequently immediate, occurring in the absence of measurable ingestion (11). This has fostered the belief that these substances act by stimulating trigeminal nociceptors (i.e., pain receptors) in the eyes, nose, and mouth (9,10). Olfaction and gustation are presumably not involved, even though repellents often have distinctive odors and tastes (2). Data from ablation studies with passerine birds (5,9) are consistent with these notions, although few substances have been tested as stimuli. We performed the present experiment to more fully explore the relationship between repellency and irritation. Electrophysiological methods were used to record the responsiveness of rat (*Rattus norvegicus*) trigeminal receptors in the oral cavity to 12 chemical repellents and ammonium chloride (1,10,12,13).

## MATERIALS AND METHODS

### *Subjects*

Eight Norway rats (*Rattus norvegicus*, laboratory strain; 4 males, 4 females; body weight: 225–550 g) served as subjects. The animals were individually caged, with free access to Purina® rat chow and water.

### *Stimuli*

Pure (HPLC grade) ortho-aminoacetophenone, meta-aminoacetophenone, para-aminoacetophenone, ortho-methoxyacetophenone, meta-methoxyacetophenone, para-methoxyacetophenone, ortho-hydroxacetophenone, meta-hydroxyacetophenone, para-hydroxy-acetophenone, 2-amino-4′,5′-methoxyacetophenone, methyl anthranilate, veratryl amine, and ammonium chloride were purchased from Aldrich Chemical Company (Milwaukee, WI). Each of the acetophenone derivatives, methyl anthranilate, and veratryl amine have served as candidate repellents in behavioral experiments (10,12,13). Ammonium chloride is a robust stimulus for rat trigeminal neurons (8,17) and was included as a positive control (3). To further characterize neurons (i.e., to distinguish thermoreceptors from polymodal nociceptors), cool water also was presented as a stimulus during each recording session.

We mixed each of the stimulants with deionized distilled water to obtain a 1% (mass/volume) mixture (ammonium chloride) or emulsion (all other chemicals). None of the emulsions was stable. Therefore, we used spectrophotometry to empirically determine saturated stimulus concentrations (Table 1).

For the chemical analyses, we prepared standards by dissolving 25 mg of stimulus in 25 ml of methanol. We mixed an aliquot (1 ml) of this solution with 1 ml of 0.2 M phosphate buffer (pH 7), and then diluted the mixture with 23 ml of distilled water. For comparison with the standard, we mixed an aliquot (1 ml) of each stimulus solution with 1 ml of 0.2 M phosphate buffer (pH 7) and then diluted the mixture with 23 ml of distilled water. After preparation of these 2 solutions, we placed an aliquot of the standard in a spectrophotometric cell and recorded the ultra-

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TABLE 1

SPECTROPHOTOMETRICALLY DETERMINED STIMULUS CONCENTRATIONS

| Stimuli | Concentrations (mg/ml) | Concentrations (M) |
|---|---|---|
| Ortho-aminoacetophenone | 6.431 | 0.047 |
| Meta-aminoacetophenone | 3.849 | 0.0285 |
| Para-aminoacetophenone | 4.073 | 0.0302 |
| Ortho-methoxyacetophenone | 3.957 | 0.0264 |
| Meta-methoxyacetophenone | 2.930 | 0.0195 |
| Para-methoxyacetophenone | 9.195 | 0.0613 |
| Ortho-hydroxyacetophenone | 2.009 | 0.0148 |
| Meta-hydroxyacetophenone | 3.215 | 0.0236 |
| Para-hydroxyacetophenone | 5.157 | 0.0380 |
| Methyl anthranilate | 3.479 | 0.0230 |
| 2-Amino-4′,5′-methoxyacetophenone | 2.178 | 0.0111 |
| Veratryl amine | 8.746 | 0.0629 |
| Ammonium chloride | 9.390 | 0.176 |

violet (UV) absorbance of the longest wavelength (AST) between 200–350 nm. Next, we placed aliquots of stimulus solutions in the cell and recorded the UV absorbance within the same wavelength range used with the standard. We determined stimulus concentrations using the following equation: $C = [(W_{ST}/25) \cdot (A/A_{ST})]$, where C was the concentration of interest (mg/ml), and $W_{ST}$ was the weight of the standard (mg).

*Procedure*

In preparation for recording, we deeply anesthetized each rat (intraperitoneal pentobarbital; males 65 mg/kg, females 50 mg/kg; urethane, 150 mg/kg), and then shaved the underside of the mandibles and the throat. We positioned each animal on its back, secured its head in a nontraumatic head holder (14), and inserted a tracheotomy tube. Surgical procedures to expose and record from the lingual branch of the trigeminal nerve are described in detail elsewhere (1). To prevent desiccation, we submerged the nerve in perfluorotributylamine oil.

Each stimulus was delivered at body temperature to prevent the spurious activation of thermal (i.e., cool- or warm-sensitive) units. Single-unit and multi-unit trigeminal neuron activity was recorded and processed using conventional methods (8).

*Analysis*

We evaluated neuronal activity during the 5 s preceding and following stimulus application in 2 2-factor repeated measures analyses of variance (ANOVA) (7). The first factor in each ANOVA was stimulus type (13 levels), and the second factor was time (2 levels). We used Tukey post hoc tests to isolate significant differences among means ($p < 0.05$) (18).

## RESULTS

*Single-Unit Activity*

Collapsed across stimuli, overall activity was significantly higher during the posttreatment period ($F = 5.3$; 12,72 df; $p < 0.001$). However, the magnitude of this increase differed among stimuli, and this difference was reflected by a significant interaction between stimulus type and time ($F = 4.85$; 12,72 df; $p < 0.001$). Post hoc tests showed that para-methoxyacetophenone and ammonium chloride were relatively weak stimuli (i.e., these stimuli elicited the smallest change in response; Fig. 1). Otherwise, there were no significant differences among stimuli. Cool water elicited increases in activity in 7 of the neurons recorded.

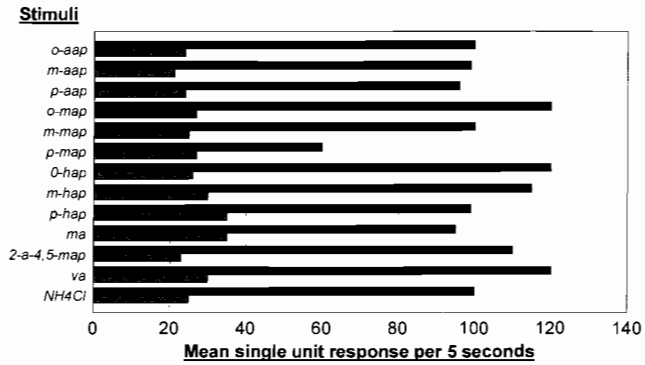


FIG. 1. Mean single-unit responding of neurons in rat lingual trigeminal nerve to 12 candidate repellents and ammonium chloride. Light bars represent responding prior to stimulation; dark bars represent responding during stimulation. o-aap, orthoaminoacetophenone; m-aap, meta-aminoacetophenone; p-aap, para-aminoaceto-phenone; o-map, ortho-methoxyacetophenone; m-map, meta-methoxyacetophenone; p-map, para-methoxyacetophenone; O-hap, ortho-hydroxacetophenone; m-hap, meta-hydroxyaceto-phenone; p-hap, para-hydroxyacetophenone; 2-a-4,5-map, 2-amino-4′,5′-methoxyacetophenone; ma, methyl anthranilate; va, veratryl amine; NH4Cl, ammonium chloride.

*Multi-Unit Activity*

Collapsed across stimuli, overall activity was higher during the posttreatment period ($F = 4.6$; 12,36 df; $p < 0.001$). As described for single units, the magnitude of this increase differed among stimuli, and this difference was reflected by a significant interaction between stimulus type and time ($F = 2.55$; 12,36 df; $p < 0.01$). Post hoc tests showed that ammonium chloride, para-methoxyacetophenone, and veratryl amine were relatively weak

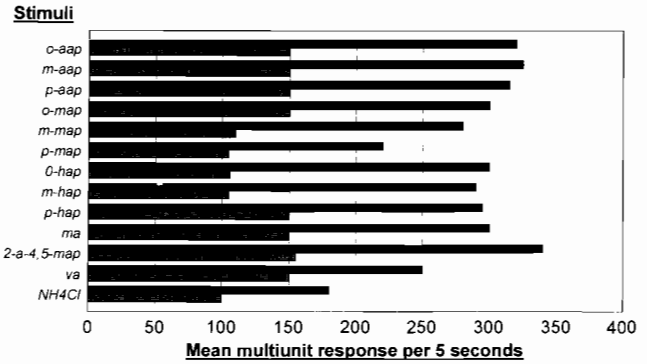


FIG. 2. Mean multi-unit responding of neurons in rat lingual trigeminal nerve to 12 candidate repellents and ammonium chloride. Light bars represent responding prior to stimulation; dark bars represent responding during stimulation. o-aap, orthoaminoacetophenone; m-aap, meta-aminoacetophenone; p-aap, para-aminoaceto-phenone; o-map, ortho-methoxyacetophenone; m-map, meta-methoxyacetophenone; p-map, para-methoxyacetophenone; O-hap, ortho-hydroxacetophenone; m-hap, meta-hydroxyaceto-phenone; p-hap, para-hydroxyacetophenone; 2-a-4,5-map, 2-amino-4′,5′-methoxyacetophenone; ma, methyl anthranilate; va, veratryl amine; NH4Cl, ammonium chloride.

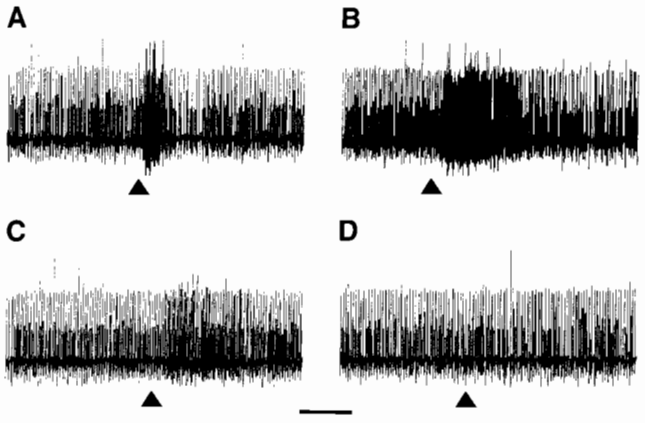


FIG. 3. Recording of a paucifiber bundle of the lingual trigeminal nerve in response to (A) m-aminoacetophenone, (B) cold (19°C), (C) ammonium chloride, and (D) p-methoxyacetophenone. Stimulus onset is indicated by the arrowheads. Time bar (bottom, center) indicates 1 s.

stimuli (Fig. 2). Otherwise, there were no significant differences among stimuli. Cool water elicited increases in activity in 4 multi-unit recordings. Sample traces of multi-unit neuronal activity are presented in Fig. 3A, B, C, and D.

## DISCUSSION

All of the candidate repellents were at least as effective as ammonium chloride, and most appeared to be stronger. This finding is consistent with the view that trigeminal receptors mediate avoidance of these substances. Also of interest was the finding that neuronal activity was predictable from behavioral avoidance exhibited toward the 12 repellents: para-methoxyacetophenone and veratryl amine are weak repellents in feeding and drinking tests (10,12,13). Electrophysiological recordings may represent a convenient and efficient method for the selection of candidate repellent compounds for evaluation in behavioral tests.

That several units responded to presentations of cool water has considerable basic significance. Specifically, the implication is that some repellent-sensitive neurons are cool-sensitive thermoreceptors or thermonociceptors, rather than polymodal nociceptors. Although polymodal nociceptors are presumed to be the primary class of receptors mediating response to chemical irritants, the present results are consistent with several lines of evidence that implicate a role for other classes of trigeminal neurons. First, aversive ionic and acidic compounds (6) are potent oral stimuli for trigeminal neurons that are not polymodal nociceptors (1,16,17). Second, cool water and aversive ammonium chloride solutions are effective stimuli in multi-unit trigeminal recordings (1). Such recordings typically contain a large number of thermoreceptors and/or thermonociceptors. Finally, stimulation of intranasal cold receptors by menthol produces a defensive depression of breathing in dogs (15).

The finding that irritants may stimulate temperature-sensitive neurons suggests the possibility of additive or synergistic interactions between chemical and thermal stimuli at the level of the receptor. This possibility is consistent with data that show that carbon dioxide is more irritating at low application temperatures (4). If synergisms reliably occur, then there are two important implications for the development of chemical repellents. First, repellents that target multiple populations of trigeminal receptors are likely to be more effective than repellents that do not. Second, it may be useful to consider the temperature at which repellents are used; both lower or higher temperatures could interact with certain repellents to produce stronger effects.

Finally, the present results have implications for basic research aimed at uncovering key molecular features of irritants (e.g., 2). Behavioral assays of repellency have been used to evaluate the relationship between structural modification of stimulus compounds and response (e.g., 2). These studies have served as the basis for models that accurately identify molecular features that predict behavioral repellency. The present results imply that these molecular features are important for trigeminal activation.

## SUMMARY

Repellent chemicals are presumed to activate trigeminal neurons (9,10). The present experiment provides electrophysiological evidence consistent with this view. All 12 stimuli evoked multi-unit and single-unit responses from the trigeminal nerve, and activity was predictable from behavioral data. Such findings support the notion that repellents are irritants, and suggest that electrophysiological methods could be useful for screening candidate repellents.

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